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From: DeBerry, Regina
Sent: Thursday, May 08, 2003 5:40 PM
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Hello,
Would you please locate the following reference:

Amano et al. The gamma-2 chain of laminin 5 is processed by BMP-1 and processing is essential to basement membrane assembly in vivo. Journal of Investigative Dermatology, (1997) Vol. 108, No. 4, page 542. Meeting Info: Annual Meeting of the Society for Investigative Dermatology Washington, DC, USA April 23-27, 1997.

Thank you.

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A HOMOZYGOUS MUTATION IN THE GENE ENCODING INTEGRIN α6 IN JUNCTIONAL EPIDERMOLYSIS BULLOSA WITH PYLORIC ATRESIA

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The α6β4 integrin heterodimer is a receptor for laminin 5, the major adhesion ligand of keratinocytes, and is the crucial component for the assembly and stability of hemidesmosomes. Recently, two distinct mutations in the β4 integrin gene (ITGB4) have been identified in a variant of junctional epidermolysis bullosa (JEB) associated with pyloric atresia (PA-JEB). In this report we describe the first mutation in the α6 integrin gene (ITGA6) in a patient with PA-JEB, and the application of mutation detection in ITGA6 gene for prenatal diagnosis in this family at risk for recurrence of the disease. RT-PCR amplification and sequence analysis of the α6 integrin transcript, showed a single base (C) deletion at position 791. This mutation (791delC) results in a frameshift and a premature termination codon 66 bp downstream (aa 268). Genomic DNA sequencing and allele specific oligonucleotide (ASO) analysis demonstrated the homozygous state of the mutation in the proband and showed both parents to be heterozygous healthy carriers for the same mutation. As the family requested a prenatal diagnosis for a new pregnancy, ASO analysis was also performed on genomic DNA from a skin biopsy obtained at 16 weeks' gestation, revealing that the fetus was a heterozygous healthy carrier. Our results indicate that, despite the ability of α6 to associate with both the β1 and β4 integrin subunits and its expression in tissues where β4 is not found, mutations in either the α6 or the β4 integrin subunit lead to the same disease, i. e. PA-JEB. In addition, this phenotype appears to be similar, although not identical, to the findings of extensive detachment of epidermis and other epithelia recently described in both α6 and β4 null mice.

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THE SUBCELLULAR DISTRIBUTION OF BULLOUS PEMPHIGOID ANTIGEN 180 (BP180) IS REGULATED BY THE β4 INTEGRIN SUBUNIT.

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Hemidesmosomes (HDs) are multiprotein receptor complexes that mediate cell adhesion and transduce signals from the extracellular matrix to the cell interior. Our knowledge of the molecular organization of HDs is incomplete. To investigate the interaction of BP180 with another hemidesmosomal component, the α6β4 integrin, we have cotransfected COS-7 cells, which lack α6β4 and BP180, with cDNAs encoding mutant forms of BP180, and of α6A and β4A. A mutant form of BP180 lacking the collagenous extracellular domain and a chimeric protein, consisting of the membrane localization sequence of K-ras fused to the cytoplasmic domain of BP180, were colocalized with α6β4 and HD1/plectin in distinct structures at cell-substrate contact sites. However, the recombinant forms of BP180 could not be coimmunoprecipitated with either α6 or β4. As an alternative approach to identify important domains within α6β4, we have transfected COS-7 cells with cDNAs for α6A and mutant forms of β4, which either lack the cytoplasmic COOH-terminal half or carry mutations of the "tyrosine activation motif". In COS-7 cells expressing α6A and the mutant forms of β4, BP180 was not colocalized with α6β4, but in most cells remained diffusely distributed over the cell surface. To confirm the involvement of the cytoplasmic domain of β4 in determing the distribution of BP180, COS-7 cells were transfected with cDNAs for α6 and a β4/β1 chimeric construct encoding the extracellular domain of β4 fused to the cytoplasmic domain of β1. In transfected cells, BP180 was not colocalized with the α6β4/1 chimera in focal adhesions. Our findings suggest that sequences contained in the cytoplasmic COOH-terminal half of β4 are critical in the regulation of the subcellular distribution of BP180. These observations provide new insights, which are relevant for the understanding of interactions involved in the assembly of HDs.

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THE ECTODOMAIN OF BULLOUS PEMPHIGOID ANTIGEN 2 (BPAG2) COMPRISES ANCHORING FILAMENTS AND ITS CARBOXYL TERMINUS LOCALIZES WITHIN THE LAMINA DENSA.

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BPAG2, type XVII collagen, is a 180 kD, type II transmembrane protein associated with hemidesmosomes (HDs) in basal keratinocytes (BKs). Acquired or inherited abnormalities in BPAG2 are responsible for the pathogenesis of several subepidermal bullous diseases. To understand how BPAG2 promotes BK adhesion to basement membrane (BM), purified IgG against a baculovirus-encoded recombinant was used to define the localization of its carboxyl-terminus in human skin by immunogold electron microscopy (IEM). A 2.1 kb BPAG2 cDNA encoding the distal ectodomain and carboxyl-terminus of BPAG2 was used in the BaculoGold Expression System to create virus that produced a 70 kD recombinant form of BPAG2 (BV4). Purified BV4 was characterized and used to raise high titer and specific rabbit IgG. Purified anti-BV4 IgG bound the epidermal side of 1M NaCl split skin and immunoprecipitated BPAG2 but no other proteins from biosynthetically radiolabeled keratinocytes. In IEM studies of pre- and post-embedded skin, the distal ectodomain of BPAG2 localized beneath HDs in BKs; there was no evidence of BPAG2 beneath melanocytes. In 1 M NaCl split skin, anti-BV4 IgG extensively bound anchoring filaments on the epidermal side of the substrate; interestingly, this staining extended along anchoring filaments to their ends. In post-embedded skin, the carboxyl-terminus of BPAG2 was localized within the lamina densa; the mean distance of 400 gold particles localizing the carboxyl terminus of BPAG2 was 41 nm beneath plasma membranes of BKs. In comparison, the localization of purified rabbit IgG directed against all subunits of laminin 5 was exclusively within the lamina densa. BPAG2 is the first molecule shown to extend from the intracellular-HD plaque of BKs to the lamina densa of human epidermal BM.

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THE 230-KD BULLOUS PEMPHIGOID ANTIGEN (BPAG1) IS EXPRESSED IN THE NERVOUS SYSTEM DURING EARLY EMBRYONIC DEVELOPMENT.

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The hemidesmosome, critical for the integrity of the skin, consists of several structural components. One of these is BPAG1 which was originally identified as one of the autoantigens in bullous pemphigoid. In this study, elucidation of the BPAG1 expression was accomplished by generating a construct consisting of 2466 bp of 5' flanking sequence of the murine BPAG1 promoter, linked to a *LacZ* reporter gene (BPA/LacZ). Transient transfections of BPA/*LacZ* into murine keratinocytes or fibroblasts with histochemical staining for β-galactosidase demonstrated keratinocyte-specific expression, consistent with Northern analysis indicating that BPAG1 is expressed only in epidermal keratinocytes. To examine the *in vivo* expression of BPAG1, a homozygous line of transgenic mice was generated using BPA/*LacZ* construct. Histochemical analysis of adult transgenic mice revealed *LacZ* expression in the basal keratinocyte layer of the epidermis, but not in the dermis. The developmental expression of BPA/LacZ was examined in embryos from day 7 to birth. BPA/*LacZ* expression was detected at day 9.5, first appearing in somite cells, but not in epithelial cells. At day 10.5, expression of LacZ was detected in the spinal and cranial ganglion cells, and in the neuro-epithelium of the spinal cord and midbrain. Also, LacZ expression was found in the cutaneous epithelium, particularly in the dorsal part of the body. This pattern of expression continues until the age of day 12.5 when expression of the gene in the nervous system stops, but is continuously detectable within the epidermis and the hair follicles. These results suggest that BPAG1 is expressed in the nervous system during the early embryonic development. The findings provide further evidence in support of the possibility that abnormalities in the expression of hemidesmosomal proteins relate to certain congenital neuro-muscular diseases associated with skin blistering, as exemplified by epidermolysis bullosa with muscular dytrophy.

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THE γ2 CHAIN OF LAMININ 5 IS PROCESSED BY BMP-1 AND PROCESSING IS ESSENTIAL TO BASEMENT MEMBRANE ASSEMBLY IN VIVO.

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Laminin 5 chains are proteolytically processed *in vitro* and *in vivo*, but the function of these cleavages are not understood nor have the enzymes responsible been identified. We have identified the newly formed N-terminal sequences following processing. The sequence of the γ2 chain cleavage site corresponds to the consensus sequence for cleavage by bone morphogenic protein 1 (BMP-1). Recombinant BMP-1 cleaves γ2 in vitro to give the predicted fragments. The α3 chain is also cleaved, but the largest fragment is larger than the *in vivo* product, indicating that the *in vivo* processing is accomplished by a separate enzyme. Inhibition of γ2 cleavage by EDTA or o-phenanthroline is consistent with the inhibition of BMP-1. BMP-1 is expressed by cultured keratinocytes as shown by RT-PCR amplification. Polyclonal antibodies made to recombinant BMP-1 immunoprecipitate BMP-1 from keratinocyte medium. Antibody reactivity with frozen sections of neonatal human foreskin show localization primarily to the dermis, but weak staining is also seen along the dermal-epidermal junction. Fetal bovine skin at a estimated gestional age of about the equivalent of the beginning of the first human trimester shows strong staining of basal keartinocytes for anti-BMP-1 antibodies. This localization of the enzyme is concomitant with basement membrane assembly. Finally, BMP-1/- mice demonstrate a failure of dermal-epidermal junction basement membrane assembly at 15 days. In total, the results demonstrate that BMP-1 is involved in laminin 5 processing in vivo, and γ2 cleavage appears to be required for basement membrane assembly. Molecular mechanisms consistent with the observations will be discussed.

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TYPE VII (ANCHORING FIBRIL) COLLAGEN EXISTS IN HUMAN INTESTINE AND SERVES AS AN ANTIGENIC TARGET IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE.

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Skin type VII (anchoring fibril) collagen (COL7) is the target for epidermolysis bullosa acquisita (EBA) autoantibodies. Of the systemic diseases associated with EBA, inflammatory bowel disease (IBD) is by far the most common (Raab et al, JAMA 1983). Initial studies suggested that COL7 was absent from intestinal tissue (Paller et al. JID, 1986, Visser et al, J Path, 1993). In this study, using freshly obtain human intestine and promptly immersing it in a cocktail of protease inhibitors, we found COL 7 in 7 out of 7 intestine samples by immunostaining and Western blot analysis of extracted gut proteins. Further, using our recently developed ELISA employing an eukaryotic recombinant non-collagenous domain (NC1) as target (Chen M et al. J Invest Dermatol - in press), we tested sera from normal humans (N = 12) and patients with biopsy proven Crohn's Disease (N = 19). Ten out of 19 sera (55%) from the Crohn's disease patients were ELISA positive while none of the normal control sera were. ELISA results were confirmed by Western blot analysis using the recombinant NC1 as antigen. From this study, we conclude that a significant number of patients with Crohn's disease have autoantibodies against the NC1 domain of type VII collagen. These data are in concordance with previous studies showing that Crohns Disease is the most frequently associated systemic illness with EBA.